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Atrial arrhythmogenesis in catecholaminergic polymorphic ventricular tachycardia – is there a mechanistic link between sarcoplasmic reticulum Ca²⁺ leak and reentry?

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Catecholaminergic polymorphic ventricular tachycardia (CPVT) is caused by mutations in the genes encoding the cardiac ryanodine receptor channel (*RyR2*) or the major sarcoplasmic reticulum (SR) Ca²⁺ buffer calsequestrin-2 (*Casq2*) (Leenhardt et al., 2012). Traditionally, CPVT has been described as a bi-directional or polymorphic ventricular arrhythmia occurring during conditions of increased sympathetic tone, which may originate from ectopic activity due to abnormal (sub)cellular Ca²⁺ handling (Leenhardt et al., 2012). Recent studies, however, have suggested that CPVT mutations in *RyR2* are also associated with atrial arrhythmias. In this edition of *Acta Physiologica*, King *et al.* (King et al., 2012) show for the first time that atria from mice homozygous for the CPVT-associated mutation P2328S in *RyR2* (*RyR2*^{S/S}) have a reduced conduction velocity (CV) and lower maximum rates of action potential (AP) upstroke velocity compared to wild-type (WT) mice. Moreover, these parameters correlated strongly with arrhythmia susceptibility suggesting a potential novel arrhythmogenic mechanism due to *RyR2* mutations.

Atrial fibrillation (AF) is the most common cardiac arrhythmia and increases cardiac morbidity and mortality. AF is a complex disease generally occurring subsequent to advanced age and/or structural heart disease, both of which create a vulnerable substrate for AF development (Wakili et al., 2011). Current therapeutic approaches have moderate efficacy and are associated with substantial risks of side-effects including proarrhythmia, which makes the development of novel mechanism-based therapeutics crucial (Dobrev and Nattel, 2010, Dobrev et al., 2012). Conceptually, AF is thought to be mediated by ectopic (triggered) activity and reentry. Ectopic activity is generally implicated in the initiation of

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AF, whereas reentry has been suggested as the main determinant of its maintenance. When AF is initiated, additional AF-related remodeling occurs, further promoting AF maintenance and the progression from paroxysmal to persistent/permanent AF (Wakili et al., 2011). This complexity in the mechanisms precludes a clear distinction between causes and consequences of AF in patients. Interestingly, AF can also develop in structurally normal hearts due to genetic predisposing factors ('lone' AF). Cases of 'lone' AF and the experimental models they inspired have helped to determine causative factors promoting AF.

Recent research in several genetic mouse models has provided substantial evidence that RyR2 dysfunction and abnormal SR Ca^{2+} release are important causative factors in the development of AF. Mice lacking the RyR2-stabilizing protein FKBP12.6 were susceptible to pacing-induced AF and showed increased SR Ca^{2+} leak and spontaneous SR Ca^{2+} release events (SCaEs) (Sood et al., 2008, Li et al., 2012). Several studies, including the one by King *et al.* in this issue, have shown that mice with CPVT mutations also have a higher susceptibility to pacing-induced AF and that this is associated with abnormal Ca^{2+} handling in atrial myocytes (Chelu et al., 2009, Shan et al., 2012, King et al., 2012). Moreover, mice with a genetic substitution of the Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII) phosphorylation site S2814 on RyR2, resulting in mimicked constitutive phosphorylation (RyR2-S2814D), showed a similar phenotype (Voigt et al., 2012), suggesting a critical role for CaMKII-dependent RyR2 phosphorylation in AF. The involvement of CaMKII mediated phosphorylation of RyR2 in CPVT is likely since ventricular tachycardias only occur at faster heart rates or following β -adrenergic stimulation (Sumitomo et al., 2003). Moreover, these findings are consistent with increased CaMKII activity, CaMKII-dependent RyR2 dysfunction, increased SR Ca^{2+} leak and SCaEs observed in human atrial myocytes from patients with chronic AF (Voigt et al., 2012). The fact that in all mouse models AF can be induced in the absence of structural heart disease suggests that RyR2 dysfunction alone can have a primary causative role in the development of AF.

Although the exact mechanisms of AF initiation due to Ca^{2+} -handling abnormalities remain incompletely understood, it is generally believed that diastolic RyR2-mediated SCaEs cause a $\text{Na}^+/\text{Ca}^{2+}$ -exchanger (NCX)-mediated transient inward current resulting in delayed afterdepolarizations (DADs) of the membrane potential, likely contributing to atrial ectopic activity (Wakili et al., 2011, Voigt et al., 2012) (Figure 1, **left side**). In agreement, RyR2^{S/S} mice showed DADs, ectopic activity and atrial tachyarrhythmias (King et al., 2012).

Much less is known about the mechanisms that sustain AF through abnormal Ca^{2+} -handling (Nattel and Dobrev, 2012). For reentry to be sustained, all points in the reentrant path need to become excitable in time for the reentrant impulse. As such, reentry is principally determined by the distance an impulse travels within a single refractory period, i.e., the wavelength (Wakili et al., 2011). When wavelength decreases due to shortening of the effective refractory period or due to reduced CV, reentry is more likely to be sustained. CV is largely determined by the tissue structure (including the composition of the extracellular matrix), the number and location of gap-junctions underlying the electrical connection between myocytes, and the properties of the voltage-gated Na^+ current (I_{Na}) needed to overcome the electrotonic load of the surrounding myocardium.

The study by King *et al.* (King et al., 2012) highlights novel arrhythmogenic consequences of abnormal Ca^{2+} handling resulting from CPVT mutations which may play a critical role in AF maintenance. Specifically, they found a reduced upstroke velocity of monophasic APs, inter-atrial conduction delays and slowed epicardial CV in structurally normal $\text{RyR2}^{\text{S/S}}$ hearts, strongly suggesting Ca^{2+} -dependent alterations in atrial Na^+ channels and/or gap-junctions which may promote functional reentry (Figure 1, **right side**).

The exact mechanisms underlying the reduced atrial CV in $\text{RyR2}^{\text{S/S}}$ mice are currently unknown. The reduced upstroke velocity of the monophasic AP suggests that the availability of voltage-gated Na^+ channels may be impaired resulting in reduced I_{Na} . Several studies have indicated that intracellular Ca^{2+} can exert complex regulatory effects on I_{Na} . The increased SR Ca^{2+} leak in CPVT mice may result in increased CaMKII activity, particularly in combination with cardiac pacing (Chelu et al., 2009), which can alter cardiac Na^+ channel function. The results depend on species, isoform, and activation method (acute versus chronic CaMKII overexpression), but in general a CaMKII-dependent gain-of-function of late (persistent) I_{Na} has been reported, whereas evidence for both gain- (Aiba et al., 2010) and loss-of-function (Wagner et al., 2006) of peak I_{Na} exists. These inconsistencies make it difficult to determine whether CaMKII-dependent modulation of peak I_{Na} can contribute to the observed conduction slowing, although increased late I_{Na} appears inconsistent with the unaltered APD observed by King et al. in $\text{RyR2}^{\text{S/S}}$ mice. On the other hand, Casini *et al.* (Casini et al., 2009) have shown that intracellular Ca^{2+} can directly inhibit I_{Na} without affecting channel gating, likely due to permeation block. Interestingly, Ca^{2+} -dependent activation of the protein phosphatase calcineurin has also been shown to result in a strong reduction in I_{Na} , a process that has been suggested to involve activation of Ca^{2+} -dependent protein kinase-C (PKC) isoforms and modulation of channel trafficking (Abriel, 2007).

In contrast, PKC-dependent regulation of gap-junction proteins has been associated with improved intercellular communication whereas dephosphorylation of gap-junction proteins may be involved in lateralization of gap-junctions and conduction abnormalities in atrial fibrillation, although this may be isoform dependent (Dobrev et al., 2012). However, several studies have suggested that intracellular Ca^{2+} can indeed reduce cellular communication via gap-junctions (Maurer and Weingart, 1987).

Ca^{2+} -dependent regulation of other ion channels/transporters may also affect CV. For example, Ca^{2+} -dependent inhibition of inward-rectifier K^+ current (I_{K1}) has been suggested (Fauconnier et al., 2005), which could, similar to Ca^{2+} -dependent stimulation of the Na^+ - Ca^{2+} exchanger, indirectly reduce I_{Na} availability by depolarizing the resting membrane potential. However, since atrial ERP was unaltered in $\text{RyR2}^{\text{S/S}}$ mice and I_{K1} is *increased* in a Ca^{2+} -dependent manner (mediated by a calcineurin, nuclear factor of activated T-cells (NFAT) and microRNA-26-dependent regulation of Kir2.1) in chronic AF (Nattel and Dobrev, 2012), the contribution of this potential mechanism appears unlikely. The relative contributions of Na^+ channels, gap-junctions and other processes to the phenotype identified here, as well as the underlying molecular mechanisms in different regions will likely be addressed in future studies.

The present article has several implications. Although the authors made use of homozygous RyR2^{S/S} mice, which contrasts with the autosomal-dominant inheritance of RyR2 mutations in CPVT patients, similar results were obtained in WT mice treated with caffeine, which promotes RyR2 opening. A programmed electrical stimulation protocol with progressively shorter S1–S2 intervals was used to determine the susceptibility to AF in WT and RyR2^{S/S} mice. Interestingly, similar AP upstroke velocities and CVs were obtained in both WT and RyR2^{S/S} mice for the S1–S2 interval that induced sustained atrial arrhythmias; however, sustained atrial arrhythmias could be induced at a much longer S1–S2 interval in RyR2^{S/S}, indicating increased susceptibility. The fact that caffeine was able to reproduce the phenotype in the current study opens the possibility to validate these findings in large-animal AF models with Ca²⁺-handling properties more similar to those in humans.

The class IC antiarrhythmic drug flecainide has been shown to have potential for the treatment of CPVT (Leenhardt et al., 2012). The exact antiarrhythmic mechanisms remain the topic of discussion and may involve reduced atrial excitability due to I_{Na} inhibition as well as direct inhibition of RyR2, reducing the occurrence of SCaEs. The Ca²⁺-dependent inhibition of I_{Na} in CPVT could therefore potentially also have antiarrhythmic effects by reducing atrial excitability (Figure 1, dashed green line). On the other hand, the conduction slowing in RyR2^{S/S} mice was quantitatively similar to that observed in heterozygous Na⁺ channel Na_v1.5-subunit knock-out (SCN5A^{+/-}) mice, or WT mice treated with flecainide, pointing to the possibility that drugs which further compromise CV or excitability may be even proarrhythmic. These hypotheses need experimental verification in subsequent work.

Of note, in a parallel study, the authors observed similar conduction abnormalities in the ventricles of RyR2^{S/S} mice but only following provocation with isoproterenol and caffeine (Zhang et al., 2012), whereas the atrial CV slowing observed here did not require adrenergic stimulation (King et al., 2012). These findings are in line with the increased susceptibility to Ca²⁺-handling abnormalities in atria versus ventricles reported recently (Shan et al., 2012). Ventricular arrhythmias in CPVT have been suggested to originate from Purkinje cells which also have a higher incidence of abnormal SCaEs and DADs than ventricular myocytes (Kang et al., 2010). The interface between Purkinje fibers and the ventricular myocardium is characterized by a small source and large electrotonic sink, suggesting that conduction abnormalities such as those identified here (King et al., 2012) may be particularly pronounced in this region and contribute to the arrhythmogenic role of the Purkinje system in CPVT.

Taken together, this study (King et al., 2012) confirms that CPVT mutations may present as a form of ‘lone’ AF and that abnormalities in SR Ca²⁺ handling can have a causative role in AF. Importantly, this study shows that CPVT mutations not only increase the susceptibility to ectopic (triggered) activity, but also create a vulnerable substrate characterized by slow conduction which is expected to favor reentry, thereby adding to our understanding of the complex roles of abnormal Ca²⁺ handling in atrial arrhythmogenesis.

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Figure 1.

Role of RyR2 dysfunction in AF. The RyR2-P2328S mutation can cause spontaneous SR Ca²⁺ release events (SCaEs), generating a transient inward current (I_{ti}) and delayed afterdepolarizations (DADs) which can contribute to ectopic activity (left). Ectopic activity can trigger reentry in a vulnerable substrate or, when occurring repetitively, can maintain AF as a “driver”. In addition, King et al. (2012) show that RyR2-P2328S causes Ca²⁺-dependent reductions in conduction velocity (CV), likely through modulation of I_{Na} and gap-junctions, creating a vulnerable substrate for reentry (right). SERCA2a: SR Ca²⁺ ATPase; PLN: phospholamban; I_{Ca,L}: L-type Ca²⁺ current; I_{NCX}: Na⁺/Ca²⁺ exchange current. Elements from ‘Servier Medical Art’ were used in the design of this figure.